D2

11 ~~24~~. (Twice amended) Embryonic stem cells according to claim 1 ~~12~~ further comprising an inducible promoter operatively associated with a recombinase coding sequence and a transcriptionally active selectable marker flanked by two recombinase recombination target sites in the genome of the stem cells.

D3

20 ~~26~~. (Thrice Amended) Non-human mammalian embryonic stem cells comprising a germline-specific promoter operatively associated with a recombinase coding sequence and a transcriptionally active selectable marker flanked by two recombinase recombination target sites in the genome of the stem cells.

D4

21 ~~28~~. (Thrice Amended) A method for excision of the transcriptionally active selectable marker from the embryonic stem cells of claim 20 ~~26~~, said method comprising:

passaging the genome derived from said embryonic stem cells through gametogenesis, wherein said passaging causes excision of the transcriptionally active selectable marker.

D5

12 ~~32~~. (Thrice Amended) A method for the production of recombinant alleles in a transgenic non-human animal, said method comprising:

introducing a nucleic acid fragment flanked by at least two recombinase recombination target sites into mammalian embryonic stem cells of claim 1 ~~12~~; and

passaging the genome derived from said embryonic stem cells through gametogenesis to obtain a transformed gamete; and

obtaining progeny from the transformed gamete, thereby producing a transgenic non-human animal having a recombinant allele therein.

~~35~~. (Thrice Amended) A method for the production of recombinant alleles in a rodent, said method comprising:

introducing a nucleic acid fragment flanked by at least two recombination target sites into embryonic stem cells of claim ~~26~~ 20, wherein said cells are rodent cells,

passaging the genome derived from said embryonic stem cells through gametogenesis without causing recombination of the recombination target sites,

producing offspring resulting from crossing the genome of a gamete produced by the gametogenesis with the genome of a wild type rodent,

whereby the nucleic acid fragment is inserted into the genome of the offspring and produces the recombinant allele therein.

~~40~~ 31. (Thrice Amended) A method for the production of recombinant alleles, said method comprising:

introducing at least one nucleic acid construct into the genome of mammalian embryonic stem cells,

wherein said at least one nucleic acid construct comprises a germline-specific promoter operatively associated with a recombinase coding sequence, a nucleic acid fragment flanked by a first pair of recombination target sites and a selectable marker flanked by a second pair of recombination target sites,

passaging the genome derived from embryonic stem cells selected for expression of the marker through gametogenesis to obtain a transformed gamete; and

crossing the genome of the transformed gamete with the genome of a wild type animal, thereby obtaining first generation progeny wherein the marker is excised in the germline.

~~41~~ 32. (Amended) A method according to claim ~~40~~ 31 wherein said first pair of recombination target sites is recognized by a recombinase which is expressed under the control of a germline-specific promoter and said second pair of recombination target sites is recognized by a recombinase which is expressed under the control of an inducible promoter or a tissue specific promoter.

No 43. (Thrice Amended) A method for the conditional assembly of functional gene(s) for expression in eukaryotic cells by recombination of individual inactive gene segments from one or more gene(s) of interest,

wherein each of said segments contains at least one recombinase recombination target site, and wherein at least one of said segments contains at least two recombinase recombination target sites,

said method comprising:

introducing said individual inactive gene segments into a mammalian embryonic stem cell of claim 42, wherein recombinase is expressed, thereby producing a DNA which encodes a functional gene of interest, the expression product of which is biologically active, upon passage of the genome derived from said embryonic stem cells through gametogenesis.

D8

34
44. (Thrice Amended) A method for the generation of recombinant non-human animal, said method comprising:

combining a nucleic acid construct comprising a germline-specific promoter operatively associated with a recombinase coding sequence with host pluripotential ES cells derived from early preimplantation embryos,

introducing these embryos into a host female, and

allowing the derived embryos to come to term such that a recombinant non-human animal is thereby produced by operation of the recombinase upon passage of the genome derived from the embryonic stem cell through gametogenesis.

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17 1
49. (Amended) The cells according to claim 42 wherein the non-human mammalian embryonic stem cell is a rodent cell.

D9

18 17
50. (Amended) The cells according to claim 49 wherein the rodent is a mouse.

19 1
51. (Amended) The cells according to claim 42 wherein the non-human mammalian embryonic stem cell is a livestock stem cell.